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Occurrence of *Cryptosporidium suis* and *Cryptosporidium scrofarum* on commercial swine farms in the Czech Republic and its associations with age and husbandry practices

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Abstract

From 2009 to 2011, the occurrence of *Cryptosporidium* spp. was investigated on 22 farms in the Czech Republic. A total of 1,620 individual faecal samples of pigs of all age categories (pre-weaned, starters, pre-growers, growers, and sows) were evaluated for presence of *Cryptosporidium* spp. by standard microscopy and molecular tools. Genotyping was done through PCR amplification and characterization of the SSU rRNA (species-specific protocols) and GP60 loci. *Cryptosporidium* spp. was found on 16 of 22 farms with a range 0.9–71.4 %. Overall, 194 (12 %) specimens were positive by microscopy and 353 (21.8 %) by PCR. While RFLP and direct sequencing of the PCR-amplified products showed presence of *Cryptosporidium suis* (142),

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Cryptosporidium scrofarum (195), *Cryptosporidium muris* (3) and 13 samples had mixed infections with *C. suis* and *C. scrofarum*, species-specific molecular tools identified *C. suis* (224), *C. scrofarum* (208), *Cryptosporidium parvum* subtype IIa A16G1R1b (1), and *C. muris* (3). In addition, a total of 82 pigs had concurrent infections with *C. suis* and *C. scrofarum*. The analysis by age showed that *C. suis* was primarily detected among pre-weaned, whereas *C. scrofarum* was mostly detected among starters, especially those weaned at a younger age. Moreover, *C. scrofarum* never has been detected in animals younger than 6 weeks of age. Also, piglets weaned at 3 weeks of age were twice more likely to be infected with *C. scrofarum* than piglets weaned at an older age. Pigs raised on straw bedding were more likely to have *Cryptosporidium* than pigs raised on slats/slurry systems. The infections with different species were not associated with loose faeces or intensity of oocyst shedding, even when comparing different age groups.

Introduction

Cryptosporidia are ubiquitous protozoans that infects all classes of vertebrates (fish, amphibians, reptiles, birds and more than 150 species of mammals) including humans (Smith et al. 2007; Fayer 2008; Chalmers and Giles 2010; Fayer 2010; Wang et al. 2010; Hadfield et al. 2011). These parasites are important due to their public health impact (serious waterborne outbreaks), the potential life-threatening nature of infection in immunocompromised patients, and economic losses caused by these pathogens in livestock (Plutzer and Karanis 2009). The life cycle of *Cryptosporidium* involves both asexual and sexual reproduction completed within an individual host (monoxenous life cycle) (Smith 2008). *Cryptosporidium* oocysts are resistant to standard chlorination, may prevail in water environments, and some species have the ability to infect various animals species. Cryptosporidia are recognized as important parasitic protozoa causing asymptomatic to severe intestinal infections of animals and humans, depending on various factors including the immunological capabilities of their host (Vítovec et al. 2006; Hamnes et al. 2007; Langkjaer et al. 2007; Zintl et al. 2007; Armson et al. 2009; Ryan and Xiao 2009; Chen et al. 2011; Budu-Amoako et al. 2012).

The first cases of pig cryptosporidiosis were reported in 1977, and described *Cryptosporidium parvum* as the causative agent (Bergeland 1977; Kennedy et al. 1977). With the advent of molecular tools, swine specific cryptosporidia were described in 1999 and 2003 (Morgan et al. 1999; Ryan et al. 2003). Initial studies revealed that pigs were probably susceptible to infection of at least seven different *Cryptosporidium* species or genotypes; however, most of these species and genotypes (C. parvum, Cryptosporidium muris, Cryptosporidium tyzzeri, Cryptosporidium sp. Eire w65.5) occurred infrequently. Additionally, the potential susceptibility of pigs was demonstrated in experimental infection studies that sought animal models for propagation of different cryptosporidia (*C. parvum*, *Cryptosporidium hominis* and *Cryptosporidium meleagridis*) (Morgan et al. 1999; Ebeid et al. 2003; Ryan et al. 2003, 2004; Xiao et al. 2006; Chen and Huang 2007; Kváč et al. 2009a, c). Other species of *Cryptosporidium* have been rarely reported in pigs, such as Cryptosporidium felis, C. muris, C. tyzzeri, Cryptosporidium rat genotype, as well as an unknown *Cryptosporidium* genotype from lagoons of pig slurry (Jenkins et al. 2010).

Cryptosporidium suis (previously known as pig genotype I) and *Cryptosporidium scrofarum* (previously known as pig genotype II) are commonly detected in pigs, and have been shown to be host specific (Morgan et al. 1998; Enemark et al. 2003; Guselle et al. 2003; Ryan et al. 2003, 2004; Xiao et al. 2006; Hamnes et al. 2007; Langkjaer et al. 2007; Suárez-Luengas et al. 2007; Johnson et al. 2008; McCarthy et al. 2008; Vítovec et al. 2006; Kváč et al. 2013). Natural porcine cryptosporidiosis has then been reported worldwide with the infection rates ranging between 0 and 87.5 % (Quílez et al. 1996; Chen and Huang 2007; Hamnes et al. 2007; Langkjaer et al. 2007; Suárez-Luengas et al. 2007; Yatswako et al. 2007; Zintl et al. 2007; Johnson et al. 2008; Armson et al. 2009; Kváč et al. 2009a, c; Ryan and Xiao 2009; Budu-Amoako et al. 2012; Chen et al. 2011; Jeníková et al. 2011; Nguyen et al. 2012a).

It has been proposed that *C. suis* more frequently infects pre-weaned pigs, whereas *C. scrofarum* is usually detected in older animals (Langkjaer et al. 2007; Johnson et al. 2008; Kváč et al. 2009a, c). This hypothesis was based on results of genus-specific molecular tools that allowed the detection of mixed infections. In 2011, Jeníková et al. (2011) reported a high number of co-infections in post-weaned pigs using species-specific primers; their findings are later confirmed by Němejc et al. (2012). The hypothesis that *C. scrofarum* is age-specific can be paralleled to the predominance of *Cryptosporidium bovis* and *Cryptosporidium ryanae* in post-weaned calves (2–11 months) (Santín et al. 2007). The age specificity of *C. scrofarum* was definitely confirmed by Kváč et al. (2013).

The swine production has become highly specialized and industrialized and its production volume has steadily increased over the last decades. In general, pork production has undergone centralization. Swine housing systems vary enormously all over the world, but generally, pig management systems can be divided into slatted or concrete floors, where the slurry is collected to minimize contact with the pigs, straw-based bedding, or a combination of both. These different variants occur in the Czech Republic and may have an impact on the presence of *Cryptosporidium* infections.

The purpose of the present study was to determine the prevalence of *Cryptosporidium* infection in farmed pigs of all different age categories, bred under various management systems, using standard parasitological methods, and PCR-based genus-broad and species-specific molecular tools. In addition, data on husbandry factors that may impact the occurrence of *Cryptosporidium*, such as housing systems, age of animals, and age at weaning were evaluated. Furthermore, the prevalence of *Cryptosporidium* spp. on farms and its impact on the observation of loose faeces were also assessed.

The present report is perhaps the most comprehensive survey of cryptosporidiosis in pigs, including statistical associations between infections and age, housing systems, infection intensity and consistency of faeces.

Material and methods

Farms and animals

The research was performed from 2009 to 2011 on 22 pig farms throughout the Czech Republic. The farms were selected randomly without previous knowledge of parasitological

status and the selection represented all of the most frequent husbandry management systems used in the Czech Republic (Table 1). All of the farms were exclusively for pork production.

The farm flooring systems were classified as follows: (1) Slurry collected either by using (1A) *SLATTED FLOOR*, whole breeding area was covered with fully slatted floor or 1/3 of the floor was slatted and the rest was concrete. In these conditions, the slurry falls through the slatted floor; (1B) *CONCRETE FLOOR*, where pigs were kept on concrete floors with no bedding. Faeces were cleared twice a day using various mechanical systems, varying from manual removal in small farms to fully automated systems; (2) *STRAW BEDDING SYSTEMS*, where animals were kept in pens with concrete floors covered with straw bedding, that was usually changed once or twice a week.

The classification by age was as follows: (1) pre-weaned piglets (from birth to the fifth week of age or weaning, whichever came first); (2) starters (from the sixth (or weaning) to the twelfth week of age); (3) pre-growers (pigs from the thirteenth to the fifteenth week of age); (4) growers (fattening pigs from the sixteenth week of age to slaughter); and (5) sows (mothers of the piglets). Age of weaning varied from 3 to 5 weeks of age (Table 1).

Sample collection and examination

Only fresh faecal samples, were collected from the floor immediately after defecation, and individually placed into plastic tubes without fixatives. The faecal consistency (loose if it took the form of the container and solid if it maintained its original shape) was noted at the time of sampling. No repeated analyses of the same animals were included in the survey to prevent cumulative prevalence. Samples were stored at 4 °C and analysed within 24 h. *Cryptosporidium* spp. in all samples were identified on the basis of oocyst morphology using the aniline–carbol–methyl violet staining method (Miláček and Vítovec 1985) using a light microscope at a magnification of ×1,000. The infection intensity was determined from the microscopic examination as number of oocysts per gram (OPG) according to Kváč et al. (2007).

For the PCR-based methods, 200 mg of faecal sample from each specimen was processed for DNA extraction through an initial homogenization by bead disruption using a FastPrep-24 instrument (Biospec Products, Bartlesville, OK, USA) for 60 s at a speed 5.5 m/s. Total DNA was then extracted using the QIAamp® DNA Stool Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The extracted DNA was kept frozen at −20 °C until used for genotyping.

PCR amplification and analyses used three different approaches: (1) use of genus-broad primers that allowed the identification of species and genotypes, (2) primers specific for *C. suis*, *C. scrofarum* and gastric cryptosporidia of mammals (*C. muris* and *Cryptosporidium andersoni*), and (3) primers to amplify the GP60 locus from *C. hominis* and *C. parvum*.

Genus-broad PCR—*Cryptosporidium* species and genotypes were determined by a two-step nested PCR protocol amplifying a fragment of the 18S rRNA gene of *Cryptosporidium* spp. using genus-specific primers. PCR was followed by digestion with the restriction

enzymes *Ssp*I and *Vsp*I as previously described Jiang et al. (2005) and direct sequencing of products Xiao et al. (2001).

Species-specific PCR—To distinguish *C. suis* and *C. scrofarum*, we used a nested PCR amplifying a fragment of the 18S rRNA gene as previously described by Jeníková et al. (2011). For detection of co-infections of intestinal and gastric species/genotypes a novel gastric cryptosporidia specific secondary forward primer was designed (5′-TAGATATTGTTCCAATGAGC-3′). Consensus primers were designed after comparing several partial 18S rRNA gene sequences for each *Cryptosporidium* species. Primary PCR and secondary reverse primer including PCR conditions were similar as previously designed Jiang et al. (2005).

Detection of co-infections with C. parvum or other intestinal cryptosporidia such as C. hominis—The PCR detection and sub-typing was performed by amplification and sequence analysis of the GP60 locus. A fragment of this gene (800 to 850 bp long) was amplified by nested PCR according to Alves et al. (2003).

Positive and negative controls (*C. serpentis* for 18S rRNA, *C. meleagridis* for GP60, and *C. andersoni* for gastric specific primers) were included in each PCR amplification. The amplicons and products of PCR/RFLP analyses were electrophoresed in 2 % agarose gels with 0.2 mg/ml ethidium bromide and visualized under ultraviolet light. The secondary PCR products were sequenced using ABI BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI3130 Genetic Analyzer (Applied Biosystems), except those obtained with *C. suis* and *C. scrofarum*-specific primers. The nucleotide sequences were analysed using Chromas Pro v1.32 (www.technilysium.com.au/chromas.html) and aligned with reference sequences using ClustalX (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/). Sequences were compared with sequences of the SSU rRNA and GP60 loci available from GenBank.

Statistic analyses

All computation was carried out with programming environment R 2.15.0 and EpiInfo (TM) 3.5.3 (Centers for Disease Control and Prevention, USA). Generalized Linear Model methodology was used for statistical analysis. More specifically, logistic regression with parameters estimation based on maximal likelihood approach was used for modelling of relationship between occurrence of *C. suis* and *C. scrofarum*, respective and explanatory variables (age of the animals, age at weaning, management systems, infection intensity and diarrhoea). Analysis of Deviance for Generalized Linear Model Fits was used for identification of the main significant effects. Odds ratios were used to determine the significance of potential risk factor variables. The probability of presence of *Cryptosporidium* sp. dependent on explanatory variables we expressed as follows:

$$\widehat{\pi}_i = \frac{exp\left(\beta_1 \mathrm{age_i} + \beta_2 \mathrm{weaning_i} + \beta_3 \mathrm{management1_i} + \ldots + \beta_5 \mathrm{management3_i}\right)}{1 + exp\left(\beta_1 \mathrm{age_i} + \beta_2 \mathrm{weaning_i} + \beta_3 \mathrm{management1_i} + \ldots + \beta_5 \mathrm{management3_i}\right)}$$

The probability of presence of diarrhoea dependent on explanatory variables was expressed as follows:

$$\widehat{\pi}_i = \frac{exp\,(\beta_1 \mathrm{age_i} + \beta_2 \mathrm{weaning_i} + \beta_3 \mathrm{management1_i} + \ldots + \beta_5 \mathrm{management3_i} + \beta_6 \mathrm{infection\ \ intensity_i} + \beta_7 \mathrm{C.\ \ suis_i} + \beta_8 \mathrm{C.\ \ pig\ \ } II_i}{1 + exp\,(\beta_1 \mathrm{age_i} + \beta_2 \mathrm{weaning_i} + \beta_3 \mathrm{management1_i} + \ldots + \beta_5 \mathrm{management3_i} + \beta_6 \mathrm{infection\ \ intensity_i} + \beta_7 \mathrm{C.\ \ suis_i} + \beta_8 \mathrm{C.\ \ pig\ \ } I}$$

Results

Prevalence and genotyping of *Cryptosporidium* spp

A total of 1,620 faecal samples (527 samples of pre-weaned piglets, 370 of starters, 86 of pre-growers, 598 of growers and 39 of sows) were examined using both parasitological and molecular tools; 194 (12.0 %) were microscopically positive for *Cryptosporidium* spp. oocysts (Fig. 1). The morphologically distinct gastric cryptosporidia were not detected by microscopy examination. The PCR analysis of all faecal samples revealed the presence of *Cryptosporidium* spp. in 353 (21.8 %) of the animals. All microscopically positive samples were molecularly confirmed, an additional 159 microscopy negative samples were PCR positive (Table 1). Microscopic examination was 1.8× less effective than PCR protocols used. Compared to PCR, the successful detection of *Cryptosporidium* oocysts by microscopy decreased with the age of animals and infection intensity.

Overall, *Cryptosporidium* infection was detected on 16 of 22 examined farms. The prevalence of infection on all the farms and its distribution by age category and housing system are described in Table 1. The use of genus-broad primers targeting the 18S rRNA gene allowed the detection of *Cryptosporidium* in 353/1,620 samples. The RFLP analysis and direct sequencing of the PCR-amplified products identified that 142 samples had *C. suis*, 195 had *C. scrofarum* and 13 had mixed infections with *C. suis* and *C. scrofarum*. Additionally, two samples of pre-weaned piglets and one of starter were positive for *C. muris*.

All samples were also amplified using swine *Cryptosporidium* species-specific primers for either *C. suis* or *C. scrofarum*, and detected that 142 samples had only *C. suis*, 126 had only *C. scrofarum*, whereas 82 had both *C. suis* and *C. scrofarum* (Table 1).

PCR amplification of all samples at the GP60 locus detected only one sample with *C. parvum*. Sequence analysis determined that it belonged to subtype IIa A16G1R1b. This sample was from a starter pig that also had *C. scrofarum* (Table 1).

Prevalence and infection intensity by age categories

Cryptosporidium oocysts were detected in pre-weaned piglets, as young as 6 days old. The occurrence of *Cryptosporidium* spp. in pigs segregated by age was evaluated using microscopic and molecular data. Overall, the highest number of microscopy positive samples was detected in pre-weaned piglets (at 5 weeks of age), whereas the overall analysis showed two peaks: the first in piglets 3–5 weeks old and the second in starters from 8 to 11 weeks old (Fig. 2). The genotype analysis revealed that the first peak was almost exclusively due to *C. suis* while the second peak was associated with *C. scrofarum* and *C. suis* (Fig. 2).

Among piglets infected with *C. suis* (up to 5th week of age) there was a positive correlation with higher infection intensity (Fig. 2). Most of the microscopy positive smears were observed in this age category (108/194; data not shown).

Piglets up to 5 weeks old were infected only with *C. suis*, whereas infections with *C. scrofarum* were detected in starter pigs 6 weeks old or older, with highest frequency observed between 7 and 11 weeks of age. The use of swine *Cryptosporidium* species-specific primers allowed the identification of mixed infections. Co-infections with *C. suis* and *C. scrofarum* were identified in pigs from the 6th week of age. Other species of *Cryptosporidium* were infrequently detected: *C. muris* in three and *C. parvum* in one animal. Those findings were not related to the age of the pigs.

Logistic regression showed that with an increasing age of animals there is a decrease of prevalence of *C. suis* (p value=9.54×10^{-8}), whereas the prevalence of *C. scrofarum* is not affected (p value=0.0727). The statistical analyses also showed that infections with *C. suis* were more likely to occur in piglets up to 5 weeks of age when compared to all other pigs 6 weeks of age or older (p value=0.0000). Conversely, pigs at 6 weeks of age or older were more frequently infected with *C. scrofarum* (OR=40.81; p value=0.0000).

Effect of age of weaning in relation to occurrence of *Cryptosporidium* infection

Most farms reported that they weaned their piglets at 3 weeks of age. The analyses by genotype showed no significant differences in the occurrence of *C. suis* (Table 2). The analysis of infections with *C. scrofarum* and mixed infections showed differences in the frequency of infections by week of weaning. Risk factor analyses showed that piglets weaned at 3 weeks of age were twice more likely to be infected with *C. scrofarum* than piglets weaned at an older age (OR=2.04, p value<0.0001). Also logistic regression revealed the positive effect of later weaning on the occurrence of *C. scrofarum* (p value=0.0413). The effect of weaning time on occurrence of *C. suis* in older age categories was not detected (p value=0.0619).

Prevalence and infection intensity of *Cryptosporidium* in relation to housing and management systems

Cryptosporidium spp. was found on 16 of 22 farms with a range 0.9–71.4 % (Table 1). The lowest prevalence of both species of pig-specific *Cryptosporidium* was detected on the farms where pigs were housed on concrete floor where slurry was collected. Out of 290 animals, 14 (4.8 %) were positive for *C. suis* and 11 (3.8 %) for *C. scrofarum*. In contrast, 181 (18.8 %) and 155 (16.1 %) of pigs kept on straw bedding were positive for *C. suis* and *C. scrofarum*, respectively. While the average prevalence of *Cryptosporidium* spp. in pigs raised on the farms with slurry collection (concrete and slatted floors) was 6.9 or 12.7 %, respectively, the infection rates were higher among pigs raised using straw bedding systems (29.7 %) (Table 3). Results of logistic regression and risk factor analyses revealed the pig straw bedding housing system was statistically linked to pig cryptosporidiosis. While pigs kept on a concrete floor are less frequently infected with both species (p value=0.0356), animals under straw bedding management system are frequently infected with *C. scrofarum*

(OR=2.88; *p* value<0.0001). Anecdotally, the infections with *C. muris* and *C. parvum* were detected in animals on straw bedding only.

Infection intensity and loose/watery samples dependence

Overall, a very low percentage of faecal specimens was loose or watery (62 from 1,620). Among *Cryptosporidium*-infected pigs, only two piglets and three starters had loose/watery faeces. All of them were kept in straw bedding systems. The small number of loose stools did not allow to determine the significance of the relationship between consistency of samples and *Cryptosporidium* infections. All piglets and starters with loose/watery samples had infection intensity lower than 5,000 OPG. This relatively low intensity was detected in 83.6 % of all positive animals. Noteworthy, the 7.9 % cases with infection intensity over 100,000 OPG were asymptomatic. Pigs infected with *C. suis* alone shed significantly higher number of oocysts (*p* value=0.0137) with the mean of 150,000 OPG (range 1,000–2,300,000) compared to pigs infected with *C. scrofarum* (35,000 OPG, range 300–440,000). *C. parvum* was detected once, in a pig co-infected with *C. scrofarum*. This animal was a 7-week-old starter (data not shown) with infection intensity of 300 OPG. The infection intensity of mixed infection of *C. suis* and *C. scrofarum* had a mean of 25,000 OPG (range 300–45,000), all these infections occurred in pigs older than 6 weeks of age. Although the presence of diarrhoea does not statistically link to *Cryptosporidium* infection, the logistic regression showed the effect of age of animal, weaning age, and management system impacting the probability of occurrence of diarrhoea. Mainly age and keeping animals under slurry-based system decreased the probability of diarrhoea (*p* value=2.68×10^{-5} and 2.42×10^{-9}, respectively).

Discussion

Cryptosporidium infections are common in pigs worldwide and have been found in all age groups. The overall *Cryptosporidium* prevalence on farm level in the range of 0.9–71.4 % observed in this study is in accordance with ranges previously published from the Czech Republic (Vítovec et al. 2006; Kváč et al. 2009a, c; Jeníková et al. 2011). The worldwide prevalence at the herd level varies broadly between 1 and 100 % (Sanford 1987; Xiao et al. 1994; Quílez et al. 1996; Wieler et al. 2001; Ryan et al. 2003; Maddox-Hyttel et al. 2006; Hamnes et al. 2007; Langkjaer et al. 2007; Suárez-Luengas et al. 2007; Johnson et al. 2008; Budu-Amoako et al. 2012). These differences are primarily due to study design and sampling strategies, differences in management systems, and the age category of the examined pigs.

Although pigs have been reported to be susceptible at least to seven different *Cryptosporidium* species or genotypes, our study findings suggest that pigs are naturally infected only with *C. suis* and *C. scrofarum* (Santín et al. 2008; Featherstone et al. 2010). Like in other studies, we have also detected very few animals with *C. muris* or *C. parvum*. These species as well as C. felis, C. meleagridis, C. tyzzeri, Cryptosporidium rat genotype, and one unknown *Cryptosporidium* genotype were previously detected in faecal, slurry or lagoons samples (Xiao et al. 2006; Chen and Huang 2007; Kváč et al. 2009a; Jenkins et al. 2010). While swine infections with *C. parvum* and *C. meleagridis* were previously

accomplished through experimental infections (Vítovec and Koudela 1992; Akiyoshi et al. 2003), the natural susceptibility of pig to other species or genotypes has not been clearly studied. Although hundreds of pig samples worldwide have been genotyped, the detection of non-pig-specific cryptosporidia remains rare. The finding of rodent genotypes is highly suggestive of spurious infections. Pigs may consume the carcasses of mice or rats, a fact previously reported by Schad et al. (1987), or the slurry may become contaminated with oocyst of non-pig genotypes. Due to the presence of abundant synanthropic rodents in the farms as well as other potential hosts (poultry, calves, etc.), the occasional detection of non-pig cryptosporidia should not be surprising. Moreover, the non-susceptibility of pig to *C. muris* and *C. tyzzeri* infection was recently revealed (Kváč et al. 2012).

Pigs, like other farm animals or humans, are infected with more than one *Cryptosporidium* species or genotype. Although pigs are naturally susceptible to 3 species, cattle to 4, sheep to 6, goats to 5 and humans to 14 species/genotypes (Ryan et al. 2005a, b; Santín et al. 2007; Kváč et al. 2009a, c; Paoletti et al. 2009; Santín and Zarlenga 2009), only few reports of mixed infection have been published in former studies (Cama et al. 2006; Kváč et al. 2009c; Santín and Zarlenga 2009; Jeníková et al. 2011; Němejc et al. 2012). The hypothesis, that the low number of detected mixed infection may be due to the direct sequencing of partial products of small subunit of rRNA in combination with PCR-RFLP (Xiao 2010), was verified in the present study and previously by Santín and Zarlenga (2009) and Jeníková et al. (2011).

This study, in accordance with our previous studies, clearly demonstrated that pigs are naturally and frequently infected with both pig-specific *Cryptosporidium*. Previous limited reports of mixed infection may be explained by very low infection intensity of *C. suis* in pigs older than 6 weeks and using of genus-broad PCR. Thus, *C. scrofarum* is to a certain extent inaccurately considered to be dominant for this age category.

We found early *C. suis* infection in 6-day-old piglets. Similarly, Vítovec et al. (2006) and Xiao et al. (1994) found cryptosporidial infections in faeces of 6, respectively 7-day-old piglets. Enemark et al. (2003) described the pre-patent period of infections with *C. suis* in the range of 2–9 days. According to Kváč et al. (2013) pigs infected with *C. scrofarum* start to shed oocysts in the range of 6–7 days post infection. In accordance with previous reports, *Cryptosporidium* was most prevalent in pigs between 5 and 12 weeks of age (Sanford 1987; Guselle et al. 2003; Ryan et al. 2003; Maddox-Hyttel et al. 2006). Jeníková et al. (2011) demonstrated age specificity of *C. scrofarum* in pigs bred in one farm in the Czech Republic. Results of the present study were based on 1,620 samples originating from 22 farms with different management system. This extensive survey confirmed the hypothesis proposed by Jeníková et al. (2011) that this pig-specific species does not infect pre-weaned piglets. This result is consistent with reports of Suárez-Luengas et al. (2007), who recorded *C. scrofarum* in pigs at the age of 2–6 months only and Johnson et al. (2008), who found this genotype only in post-weaned pigs. Also Langkjaer et al. (2007) suggested an age-specific distribution of *Cryptosporidium* species and genotypes in pigs, where *C. suis* was more prevalent and predominantly detected in nursery pigs (pre-weaned), while *C. scrofarum* only in weaned piglets and older age categories. No other shedding of *C. scrofarum* was detected in any animals up to 5 week of age from the same farm. Similar results were published by

Wang et al. (2010), as they detected only 1 sample positive for *C. scrofarum* in pigs younger than 1 month. Finally, the age specificity of *C. scrofarum* was experimentally demonstrated by Kváč et al. (2013). In addition, examination of lagoons with slurry originating from nursery operation revealed very low concentration of this species.

Weaning is a critical step in the raising of pigs, and it is biologically critical for piglets on the development of their immune systems while adjusting to dietary and environmental changes. At present, it is accepted that shorter lactation lengths with weaning at an early age may help control the incidence of other specific diseases (Thompson et al. 1996). However, our findings indicate that early weaning is associated with increased risk of infections with *C. scrofarum*. The effect of weaning on the incidence of *Cryptosporidium* spp. in pigs has never been assessed before. However, e.g. Nguyen et al. (2012b) reported that pre-weaned piglets are at the highest risk for *Cryptosporidium* spp. infection (without molecular characterisation of *Cryptosporidium* species), followed by post-weaners, sows and finishing pigs. Due to lack of published reports, this hypothesis should be verified in the future studies.

The swine industry is characterized by a wide range of diverse farming systems that are affected not only by geographical environments, but also by social and cultural differences. In Europe, including the Czech Republic, two main systems of pig breeding are applied: Slurry- and straw-based systems in various combinations with slatted floor. The significantly lower prevalence of both pig-specific *Cryptosporidium* was detected in units where pigs were farmed using slurry-based technology, either slatted or concrete systems, when compared with straw-based systems. On the other hand, some previous studies showed a significantly higher *Cryptosporidium* infection rate in pigs on farms with porous concrete floors rather than on farms with fully slatted or semi-slatted floors (Xiao et al. 1994, 2004; Maddox-Hyttel et al. 2006). In summary, present and previous studies of Xiao et al. (1994), Maddox-Hyttel et al. (2006) and Nguyen et al. (2012b) revealed the effects of poor hygiene depending on the way that faeces were removed, on the frequency of *Cryptosporidium* spp. in a given farm. Our results revealed that straw bedding housing system of pigs was strongly associated with the presence of pig cryptosporidiosis.

Generally, it is believed that *Cryptosporidium* spp. can cause diarrhoea, but epidemiological evidence has linked clinical signs of cryptosporidiosis to be species/genotypes dependent. The relationship of infections with clinical symptoms, primarily diarrhoea, varies in pigs. Both *C. parvum* (calf origin) and *C. hominis* (human origin) have been reported to cause clinical cryptosporidiosis in pigs (diarrhoea) (Moon and Bemrick 1981; Moon et al. 1982; Tzipori et al. 1981, 1982; Argenzio et al. 1990; Vítovec and Koudela 1992; Ebeid et al. 2003), but no diarrhoeal outbreaks caused in these species have ever been reported in naturally infected pigs. Also, Xiao et al. (1994) and Quílez et al. (1996) observed severe and intense infections with *Cryptosporidium* spp. in weaned and fattening pigs (genotype information not available at the time of those studies). Enemark et al. (2003) described clinical anorexia and voluminous, watery diarrhoea in pigs experimentally infected with *C. suis* at 4–6 days post-infection and Mišič et al. (2003) reported that all the *Cryptosporidium* positive nursing piglets had diarrhoea. Furthermore, Sanford (1987) reported high occurrence of diarrhoea in *Cryptosporidium*-infected pigs, but note, that most of them might

have had other primary diarrhoeagenic agents. However, Nguyen et al. (2012b) showed association between the occurrence of diarrhoea and the level of *Cryptosporidium* oocyst excretion, the effect of various *Cryptosporidium* species/genotypes remain unclear due to missing of genotyping. Except for these reports, the findings from this study and most previously published literature concur that pigs naturally infected with both pig-specific species do not produce diarrhoea. This is similar to the observations of cattle naturally infected with *C. bovis* and *C. ryanae* (Sanford 1987; Xiao et al. 1994; Quílez et al. 1996; Guselle et al. 2003; Hamnes et al. 2006, 2007; Vítovec et al. 2006; Johnson et al. 2008; Kváč et al. 2009a, c; Jeníková et al. 2011; Němejc et al. 2012).

Despite relatively high prevalence of pig *Cryptosporidium* spp. worldwide and their presence in environmental water samples, sporadic cases of both *C. suis* and *C. scrofarum* in humans have been reported (Xiao et al. 2002, 2012; Cama et al. 2003; Kváč et al. 2009b). Thus, it implies that human infections caused by pig-specific species are limited and *C. suis* and *C. scrofarum* have little to no bearing on human cryptosporidiosis.

Finally, the actual impact of cryptosporidial infections on the health and productivity of swine is not fully understood. Although it appears that diarrhoea or loose faeces are not characteristics of infection, the long-term effects on growth rates or feed conversion ratios have not been evaluated. In children living in cryptosporidium endemic areas, infections with *Cryptosporidium* spp. were not always associated with diarrhoea, however, it was documented that infections were associated with stunting that resulted in significant growth faltering (Checkley et al. 1997). Based on these observations, it would be useful to determine if similar clinical effects may occur in pigs naturally infected with pig-specific *Cryptosporidium* genotypes.

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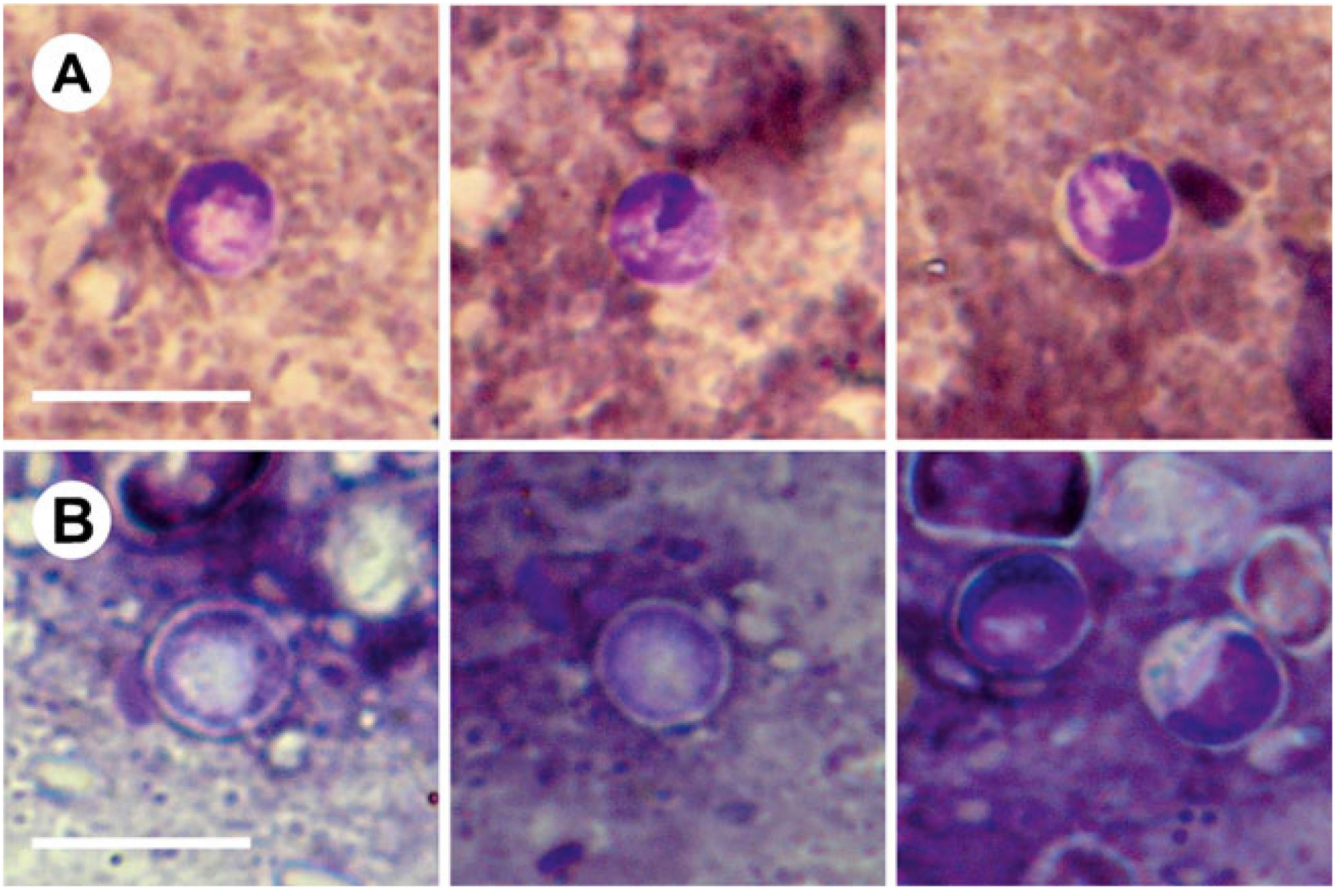

Fig. 1.
Oocyst of **a** *Cryptosporidium scrofarum* and **b** *Cryptosporidium suis* stained using the aniline–carbol–methyl violet method (Miláček and Vítovec 1985). *Bar* 10 μm

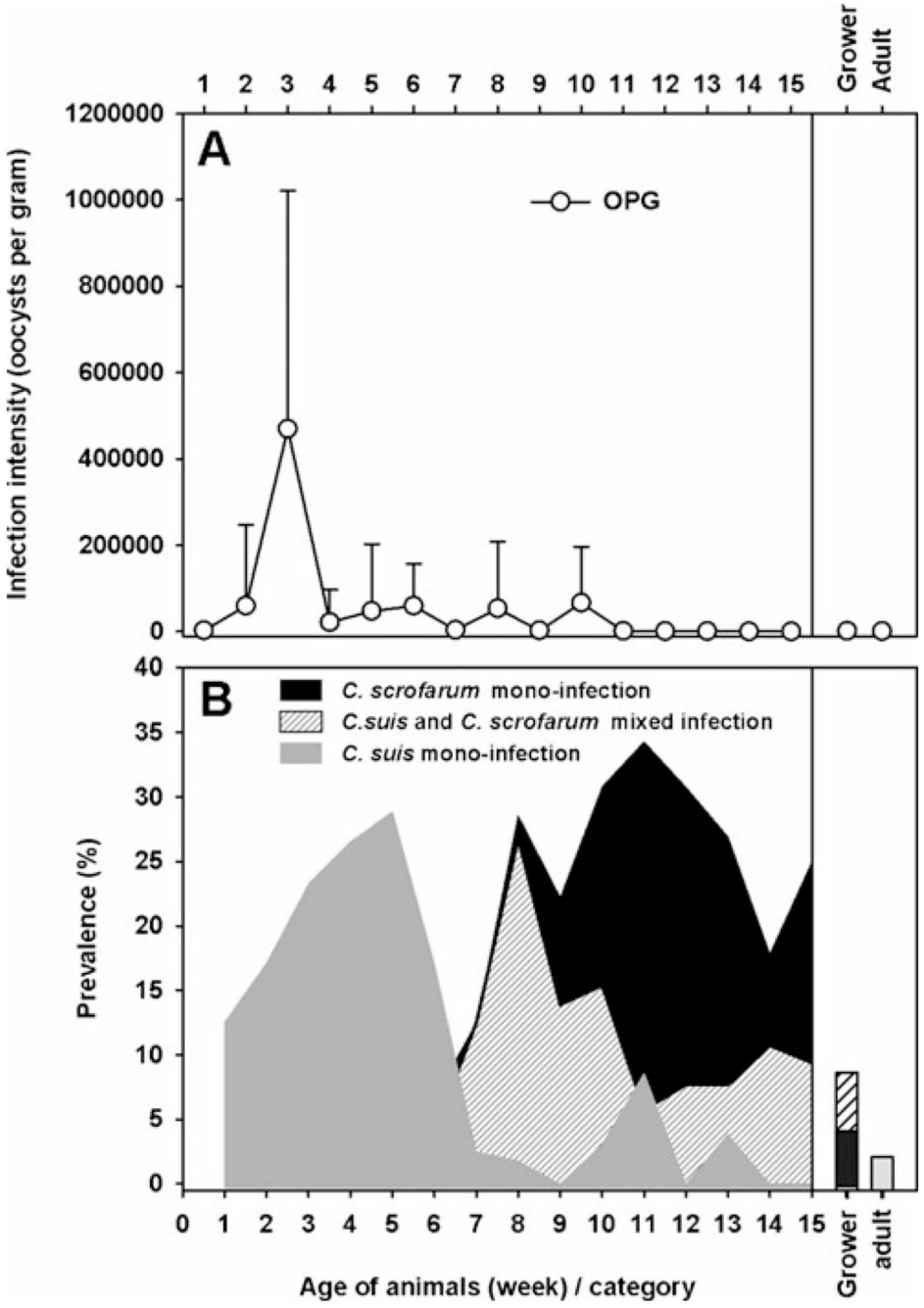

Fig. 2.

Prevalence and infection intensity of *Cryptosporidium suis* and *Cryptosporidium scrofarum* in pigs depending on age of animals. **a** Infection intensity expressed as oocysts per gram (*OPG*); **b** prevalence of *Cryptosporidium* spp. depending on age of animals based on PCR of partial 18S rRNA gene amplified using species-specific primers

Table 1

A survey of *Cryptosporidium* spp. in faecal samples on pig farms with different types of housing and management systems

Farm	Examined categories	Housing system	Weaning [weeks]	*Cryptosporidium* spp.				*C. suis*		*C. scrofarum*		*C. suis* + *C. scrofarum* mixed infection		*C. parvum*		*C. muris*	
				No. of examined samples	No. of positive samples [microscopy]	No. of positive samples [PCR]	Prevalence (PCR) [%]	No. of positive samples	Prevalence [%]	No. of positive samples	Prevalence [%]	No. of positive samples	Prevalence [%]	No. of positive samples	Prevalence [%]	No. of positive samples	Prevalence [%]
1	–,–,–,G,–	–,–,–,1,–	4	36	0	3	8.3	0	0	0	0	3	8.3	0	0	0	0
2	–,–,–,G,–	–,–,–,1,–	4	183	6	16	8.7	0	0	11	6.0	5	2.7	0	0	0	0
3	–,–,–,G,–	–,–,–,1,–	3	110	0	1	0.9	0	0	0	0	1	0.9	0	0	0	0
4	P,S,–,G,A	3,3,–,3,3	3	150	16	16	10.7	15	10.0	0	0	1	0.7	0	0	0	0
5	P,S, PG,–,–	3,3,3,–,–	3	318	56	163	51.3	42	13.2	82	25.8	39	12.3	0	0	0	0
6	P,S,–,–,–	3,3,–,–,–	4	70	20	21	30.0	18	25.7	0	0	2	2.9	0	0	1	1.4
7	–,S,PG, G,–	–,3,3,3,–	5	148	18	27	18.2	2	1.4	17	11.5	7	4.7	0	0	1	0.7
8	–,–,–,G,–	–,–,–1,–	4	28	0	20	71.4	0	0	7	25.0	13	46.4	0	0	0	0
9	–,S,–,–,–	–,2,–,–,–	5	4	0	2	50.0	1	25.0	0	0	1	25.0	0	0	0	0
10	P,S,–,–,–	2,2,–,–,–	5	32	0	0	0	0	0	0	0	0	0	0	0	0	0
11	P,S,–,–,A	3,3,–,–,3	3	138	35	36	26.1	31	22.5	1	0.7	3	2.2	0	0	1	0.7
12	P,S,–,G,–	2,1,–,3,–	4	77	13	15	19.5	13	16.9	0	0	2	2.6	0	0	0	0
13	P,S,–,–,–	3,3,–,–,–	4	93	21	21	22.6	20	21.5	1	1.1	0	0	1[a]	1.1	0	0
14	–,–,–,G,–	–,–,–2,–	3	21	0	1	4.8	0	0	0	0	1	4.8	0	0	0	0
15	–,–,–G,–	–,–,–,2–	4	76	5	5	6.6	0	0	3	3.9	2	2.6	0	0	0	0
16	–,–,–G,–	–,–,–,3–	3	10	0	0	0	0	0	0	0	0	0	0	0	0	0
17	–,–,–G,–	–,–,–,3–	5	22	0	2	9.1	0	0	1	4.5	1	4.5	0	0	0	0
18	–,–,–G,–	–,–,–,2–	3	65	4	4	6.2	0	0	3	4.6	1	1.5	0	0	0	0
19	–,–,–G,–	–,–,–,2–	4	9	0	0	0	0	0	0	0	0	0	0	0	0	0
20	–,–,–G,–	–,–,–,2–	5	4	0	0	0	0	0	0	0	0	0	0	0	0	0
21	–,–,–G,–	–,–,–,3–	4	8	0	0	0	0	0	0	0	0	0	0	0	0	0
22	–,–,–G,–	–,–,–,2–	5	18	0	0	0	0	0	0	0	0	0	0	0	0	0

Farm	Examined categories	Housing system	Weaning [weeks]	*Cryptosporidium* spp.				*C. suis*		*C. scrofarum*		*C. suis* + *C. scrofarum* mixed infection		*C. parvum*		*C. muris*	
				No. of examined samples	No. of positive samples [microscopy]	No. of positive samples [PCR]	Prevalence (PCR) [%]	No. of positive samples	Prevalence [%]	No. of positive samples	Prevalence [%]	No. of positive samples	Prevalence [%]	No. of positive samples	Prevalence [%]	No. of positive samples	Prevalence [%]
Total				1620	194	353	21.8	142	8.8	126	7.8	82	5.1	1[a]	0.1	3	0.2

Categories of pigs: *P* pre-weaned piglets; *S* starters; *PG* pre-growers; *G* growers; *A* sows; Housing and management system: 1—fully or partially slatted floor; 2—concrete floor; 3—straw bedding

[a]Mixed infection of *Cryptosporidium parvum* and *Cryptosporidium scrofarum*

Table 2

Occurrence of *Cryptosporidium* spp. infection in pigs pre- and post-weaning and risk factor analyses depending on age of weaning

Age of weaning (weeks)	Pre-weaning					Post-weaning				
	n	***C. suis***	***C. scrofarum***	***C. suis* + *C. scrofarum* mixed infection**	**Other**	***n***	***C. suis***	***C. scrofarum***	***C. suis* + *C. scrofarum* mixed infection**	**Other**
	Number of positive animals					**Number of positive animals**				
3	205	47	0	0	0	607	41	86	46	1[a]
4	157	22	0	0	0	347	29	19	25	2[a,b]
5	16	0	0	0	1[a]	288	3	21	11	0
Total	378	69	0	0	1	1242	73	126	82	3

[a] C. muris

[b] C. parvum

Table 3

Prevalence and risk factor of *Cryptosporidium* spp. occurrence depending on housing management

Housing and management system			No. of samples examined	Positive PCR							
				Monoinfection				*C. suis* + *C. scrofarum* mixed infection		Other	
				C. suis		*C. scrofarum*					
				n	%	*n*	%	*n*	%	*n*	%
Categories	P	Slatted floor	0	0	0	0	0	0	0	0	0
		Concrete floor	77	8	10.4	0	0	0	0	0	0
		Straw bedding	450	110	24.4	0	0	0	0	2[a]	0.4
	S	Slatted floor	11	5	45.5	0	0	2	18.2	0	0
		Concrete floor	20	1	5.0	0	0	1	5.0	0	0
		Straw bedding	339	14	4.1	81	23.9	44	13.0	2[a,b]	0.6
	PG	Slatted floor	0	0	0	0	0	0	0	0	0
		Concrete floor	0	0	0	0	0	0	0	0	0
		Straw bedding	86	1	1.2	20	23.3	8	9.3	0	0
	G	Slatted floor	357	0	0	18	5.0	22	6.2	0	0
		Concrete floor	193	0	0	6	3.1	4	2.1	0	0
		Straw bedding	48	2	4.2	1	2.1	1	2.1	0	0
	A	Slatted floor	0	0	0	0	0	0	0	0	0
		Concrete floor	0	0	0	0	0	0	0	0	0
		Straw bedding	39	1	2.6	0	0	0	0	0	0
Total			1620	142	8.8	126	7.8	82	5.1	4	0.2

P pre-weaned piglets; *S* starters; *PG* pre-growers; *G* growers; *A* sows;

[a] C. muris

[b] C. parvum